Tetrahedron Vol. 40, No. 23, pp. 4929 to 4935, 1984
Printed in Great Britain.

0040-4020/84 $3.00 + .00

KINETICS AND MECHANISM OF THE OXIDATION OF AMINO ACIDS BY PEROXOMONOSULPHATE

OXIDATION OF β-PHENYLALANINE, ISOLEUCINE AND THREONINE

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(*Received in UK 9 November* 1983)

Abstract—The kinetics of oxidation of amino acids (AA) by peroxomonosulphate (PMS) in the presence and absence of formaldehyde were studied. Analysis of the results shows that the rate can be represented as, at constant $[H^+]$ and in the absence of formaldehyde

$$\frac{-d[PMS]}{dt} = k_a[AA][PMS].$$

In the presence of formaldehyde and at constant $[H^+]$ the rate law is

$$\frac{-d[PMS]}{dt} = k_b[AA][HCHO][PMS] + k_c[HCHO][PMS].$$

Perusal of the kinetic results show that the formaldehyde catalysed reaction occurs $\sim 10^5$ times faster than uncatalysed and this is attributed to the formation of Schiff base. Mechanism of the reaction is discussed in terms of kinetic results.

In our earlier reports, we have discussed the kinetics and mechanism of the oxidation of amino acids by peroxomonosulphate[1] and the catalytic effect of formaldehyde.[2] In the oxidations of amino acids by PMS, valine differs from the other amino acids such as glycine, alanine, butyrine, leucine etc. The oxidation of valine by PMS follows a perfect second order kinetics at constant $[H^+]$, first order each in [substrate] and [oxidant] and there is no catalytic effect of the product; in the oxidations of other amino acids the product, aldehyde, catalyses the reaction. This is explained by the fact that the resultant product in the oxidations of amino acids forms an addition compound (Schiff base) with the parent amino acid and this Schiff base formation may not be possible in valine due to steric hindrance. In order to find out whether the steric hindrance alone is responsible for the behaviour of valine towards the oxidation by PMS and as a continuation of our programme on the construction of chemical models for the pyridoxal catalysed amino acid metabolism, we studied the kinetics and the catalytic effect of formaldehyde on the oxidation of some dietary important amino acids,[3] β-phenylalanine, isoleucine and threonine by PMS. The results are discussed in this paper.

RESULTS AND DISCUSSION

All the experiments were carried out with [amino acid] ≫ [oxidant] and the catalytic effect of formaldehyde was studied using [HCHO] ≃ [PMS]. Plots of logarithms of volume of thiosulphate consumed ($\log V_t$) versus time (Figs 1 and 2) were linear even up to 70% conversion of PMS. In the absence of formaldehyde, threonine as substrate, the plots of $\log V_t$ versus time (Fig. 1) were linear only at the initial stage of the reaction. After 2–5% conversion of PMS, the plots showed a curvature towards the x-axis. This may be due to the fact that either the product may also be oxidized or the product catalyses the reaction. Therefore k_{obs} values, for the oxidation of threonine alone, were calculated from the initial part of the kinetics where in the conversion of PMS were not more than 5%. At constant amino acid and hydrogen ion/formaldehyde concentrations the values of k_{obs} were found to be independent of the initial concentration of PMS. This shows that the rate is first order in [PMS].

The values of k_{obs}, at a fixed concentration of PMS and H^+, were found to vary linearly with amino acid concentration. Plots of k_{obs} versus [amino acid] were found to be linear passing through the origin (Fig. 3). This shows that at constant $[H^+]$, the rate equation for the uncatalysed reaction can be written as

$$\frac{-d[PMS]}{dt} = k_a[\text{amino acid}][PMS]. \quad (1)$$

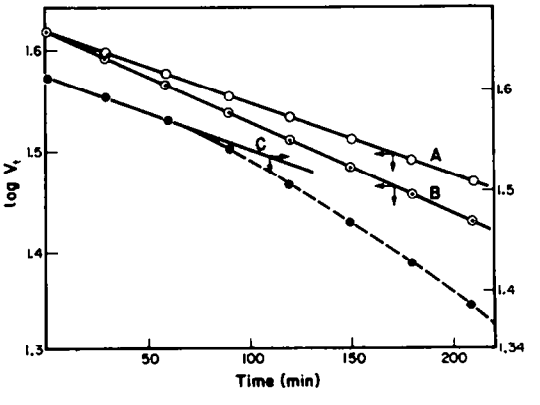


Fig. 1. Plot of $\log V_t$ versus time at 31°. A, [Phenylalanine] = 0.025 M; [oxone] = 2×10^{-3} M; $\mu = 0.25$; pH = 4.8. B, [Isoleucine] = 0.03 M; [oxone] = 2×10^{-3} M; $\mu = 0.25$; pH = 5.2. C, [Threonine] = 0.06 M; [oxone] = 2×10^{-3} M; $\mu = 0.25$; pH = 4.0.

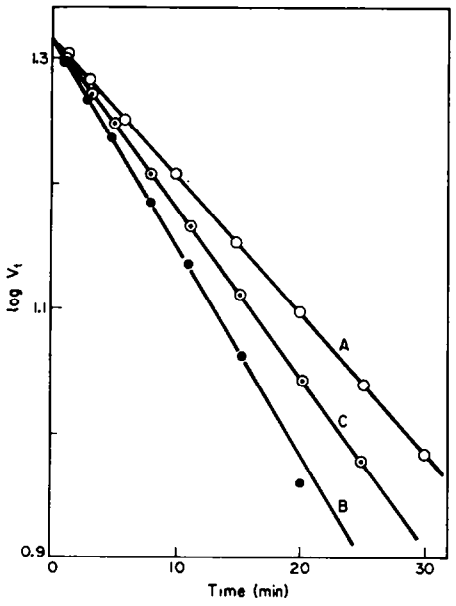


Fig. 2. Plot of log V_t versus time at 31°. A, [Phenylalanine] = 0.036 M; [HCHO] = 2.825×10^{-3} M; [oxone] = 2×10^{-3} M; $\mu = 0.25$; pH = 4.0. B, [Threonine] = 0.06 M; [HCHO] = 2.825×10^{-3} M; [oxone] = 2×10^{-3} M; $\mu = 0.25$; pH = 4.0. C, [Isoleucine] = 0.05 M; [HCHO] = 2.825×10^{-3} M; [oxone] = 2×10^{-3} M; $\mu = 0.25$; pH = 4.0.

In the formaldehyde catalysed reaction also, at constant [HCHO], [PMS] and [H^+], the values of k_{obs} were found to increase with increase in amino acids. Plots of k_{obs} versus [amino acid] were found to be linear with a definite positive intercept (Fig. 4). This clearly proves that in the formaldehyde catalysed oxidation of amino acids, the rate equation can be represented as

$$\frac{-d[PMS]}{dt} = k'_b[AA][PMS] + c[PMS]. \quad (2)$$

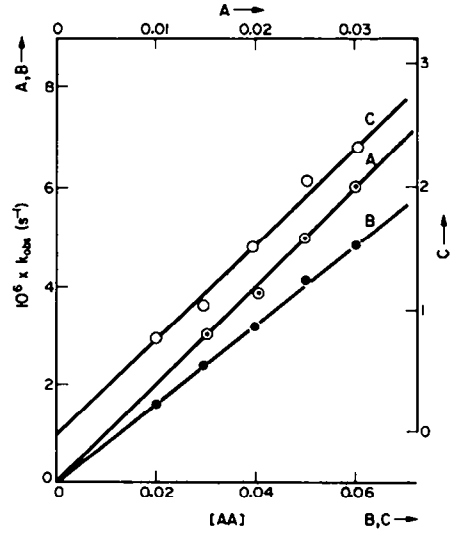


Fig. 3. Plot of k_{obs} versus [amino acid]. pH = 4.0; $\mu = 0.25$; t = 31°. A, Phenylalanine; B, isoleucine; C, threonine.

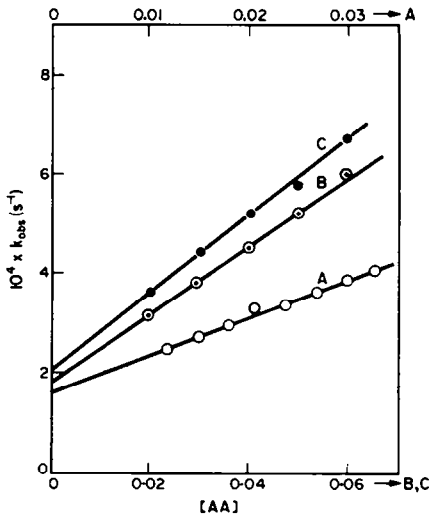


Fig. 4. Plot of k_{obs} versus [amino acid] for the formaldehyde catalysed reaction. [HCHO] = 2.825×10^{-3} M; $\mu = 0.25$; pH = 4.0; t = 31°. A, Phenylalanine; B, isoleucine; C, threonine.

Keeping [H^+] and [amino acid] constant, increase in the concentration of formaldehyde increased the values of k_{obs}. Plots of k_{obs} versus [HCHO] were found to be straight lines passing through the origin (Fig. 5). This shows that the disappearance of PMS can be expressed for the formaldehyde catalysed reaction as

$$\frac{-d[PMS]}{dt} = k'_c[HCHO][PMS]. \quad (3)$$

Comparison of Eqs (2) and (3) shows that the disappearance of PMS is of the form

$$\frac{-d[PMS]}{dt} = k_b[AA][HCHO][PMS] + k_c[HCHO][PMS]. \quad (4)$$

The values of k_{obs} were found to decrease with increase in [H^+] for all the systems and the plots of k_{obs}

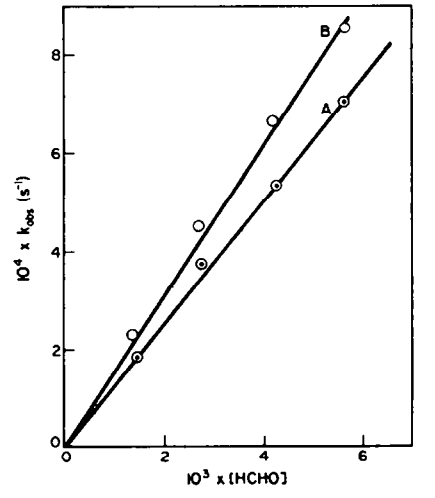


Fig. 5. Plot of k_{obs} versus [HCHO] at 31°. [AA] = 0.03 M; pH = 4.0; $\mu = 0.25$. A, Phenylalanine; B, threonine.

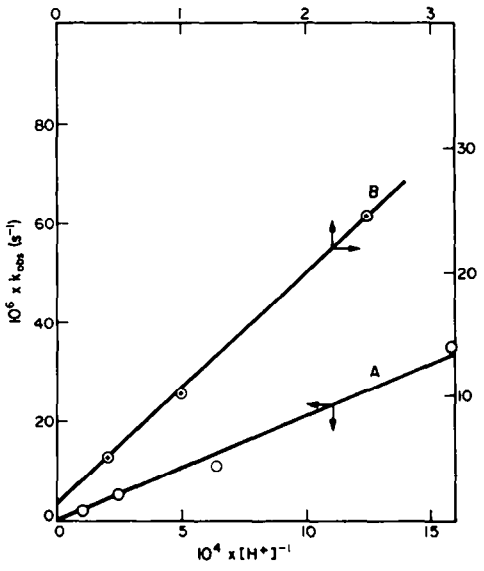


Fig. 6. Plot of k_{obs} versus $[H^+]^{-1}$. $\mu = 0.25$; t = 31°. A, [Phenylalanine] = 0.025 M; B, [Threonine] = 0.03 M.

versus $1/[H^+]$ were found to be linear with a positive slope (Figs 6 and 7). In the case of phenylalanine–PMS and isoleucine–PMS the plot of k_{obs} versus $1/[H^+]$ passes through origin and all other cases result in a positive intercept. This clearly shows that except phenylalanine–PMS and isoleucine–PMS, all other reactions proceed through two independent paths; one is inverse hydrogen ion dependent and the other is H^+ ion independent. Phenylalanine–PMS and isoleucine–PMS proceed through only the $[H^+]^{-1}$ dependent step and the second step occurs only to a negligible extent.

The change in ionic strength, over the range 0.25–0.5, showed a small increase in k_{obs} with increase in ionic strength. The reactions were studied at three different temperatures (30–45°) and from the temperature

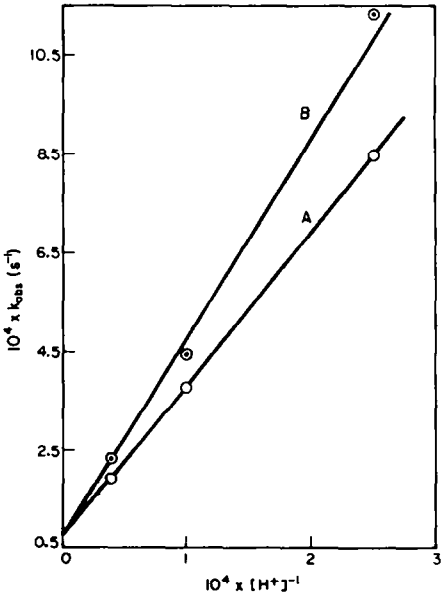


Fig. 7. Plot of k_{obs} versus $[H^+]^{-1}$ for the formaldehyde catalysed reaction at 31° and $\mu = 0.25$. [AA] = 0.025 M; [HCHO] = 2.825×10^{-3} M. A, Phenylalanine; B, threonine.

dependence of k_{obs}, the activation parameters $\Delta H^\ddagger$ and $\Delta S^\ddagger$ were calculated and tabulated. However it should be mentioned here that, since amino acid–PMS systems show different degrees of $[H^+]$ dependence, k values at a constant pH (4.0) were used to calculate $\Delta H^\ddagger$ and $\Delta S^\ddagger$. Therefore $\Delta S^\ddagger$ values are not standard entropy change values, but we hope it will serve the purpose of comparison of the three amino acid–PMS systems since all the reactions were carried out at identical conditions. Similarly in the amino acid–HCHO–PMS reaction the values used were the pseudo-first-order rate constant, k_{obs}, at constant $[H^+]$, [HCHO] and [AA] (pH = 4.0, $[HCHO] = 2.825 \times 10^{-3}$ M and [AA] = 0.03 M).

The knowledge of the reactant species that exist at the experimental conditions or involved in the reaction is essential to know the reaction mechanism. For amino acids, the following equilibria exist in acidic/alkaline solutions.

$$\mathrm{R{-}\underset{\underset{NH_3^+}{|}}{CH}{-}COOH \overset{K_1}{\rightleftharpoons} R{-}\underset{\underset{NH_3^+}{|}}{CH}{-}COO^- \overset{K_2}{\rightleftharpoons} R{-}\underset{\underset{NH_2}{|}}{CH}{-}COO^-}$$

The values of pK_1 and pK_2 for the amino acids are ~2.2 and 9.1–9.7 respectively.[4] Under our experimental conditions, namely at pH = 4.0, all the amino acids would be in the form of zwitter ions. Therefore the amino acids in its zwitter ionic form may be the reactive species.

There are a large number of references for the reaction of aldehyde with amino acid to form a Schiff base. In fact the catalytic effect of pyridoxal phosphate in the amino acid metabolism by enzymes is attributed to the Schiff base formed between the amino acid and pyridoxal phosphate.[5–7] We can therefore safely assume that the following equilibrium exists at our experimental conditions

$$\mathrm{R{-}\underset{\underset{NH_3^+}{|}}{CH}{-}COO^- + HCHO \rightleftharpoons R{-}\underset{\underset{\underset{\underset{CH_2}{\|}}{NH^+}}{|}}{CH}{-}COO^-}$$

Peroxomonosulphuric acid ($HO{-}OSO_3{-}H$) has two ionizable protons, one is the sulphuric acid proton and the other is the hydrogen peroxide proton. The pK_a value of the sulphuric acid proton lies in a high acidity region and that of hydrogen peroxide proton[8] is 9.4.

In general the reactions of peroxides are liable to acid catalysis. The present investigation is interesting in that an inverse acid dependence is observed. Since the rate was found to increase as the pH was increased and decrease as $[H^+]$ was increased and also from the nature of the k_{obs} versus $1/[H^+]$ plot leaving an intercept on the ordinate, the following mechanism for oxidation involving acid independent and inverse acid dependent paths may be proposed as

$$\mathrm{HOOSO_3^- \overset{K}{\rightleftharpoons} {}^-O{-}O{-}SO_3^- + H^+} \qquad (5)$$

$$\mathrm{R{-}\underset{\underset{NH_3^+}{|}}{CH}{-}COO^- + HCHO \overset{K_1}{\rightleftharpoons} R{-}\underset{\underset{\underset{\underset{CH_2}{\|}}{NH^+}}{|}}{CH}{-}COO^-} \qquad (6)$$

(Schiff base)

Table 1. Kinetic parameters of oxidation of amino acids by PMS at 31° and $\mu = 0.25$

	Phenylalanine	Isoleucine	Threonine
$10^6 \times k_a$ ($l\ mol^{-1}\ s^{-1}$)†	200	78	380
$10^5 \times k_1$ ($l\ mol^{-1}\ s^{-1}$)	—	—	4.7
k_2 ($l\ mol^{-1}\ s^{-1}$)	47.2	17.6	76.2
$\Delta H^{\ddagger}$ (k cal mol^{-1})†	23.0	21.4	13.6
$\Delta S^{\ddagger}$ (cal $deg^{-1}\ mol^{-1}$)†	0.0	−7.5	−30.3

† pH = 4.0; $\mu = 0.25$.

$$\text{Amino acid} + HOOSO_3^- \xrightarrow{k_1} \text{products} \quad (7)$$

$$\text{Amino acid} + {}^-OOSO_3^- \xrightarrow{k_2} \text{products} \quad (8)$$

$$\text{Schiff base} + HSO_5^- \xrightarrow{k_3} \text{products} \quad (9)$$

$$HCHO + HSO_5^- \xrightarrow{k_4} \text{products} \quad (10)$$

$$\text{Schiff base} + SO_5^{2-} \xrightarrow{k_5} \text{products} \quad (11)$$

$$HCHO + SO_5^{2-} \xrightarrow{k_6} \text{products}. \quad (12)$$

The rate law for the oxidation of amino acids in the absence of formaldehyde can be written as

$$\frac{-d[PMS]}{dt} = k_1[AA][HSO_5^-] + k_2[AA][SO_5^{2-}]$$

$$= k_1[AA][HSO_5^-] + k_2K\frac{[AA][HSO_5^-]}{[H^+]} \quad (13)$$

and therefore

$$k_{obs} = k_1[AA] + k_2K[AA]\frac{1}{[H^+]}. \quad (14)$$

Using the literature value[8] of $K = 3.98 \times 10^{-10}$, the rate constant for the oxidation by HSO_5^- (k_1) and SO_5^{2-} (k_2) are obtained and tabulated (Table 1).

Comparison of the results in Tables 1 and 2 shows that the formaldehyde catalysed reaction occurs approximately five orders of magnitude faster than the uncatalysed reaction and therefore Eqs (7) and (8) can be neglected in the oxidation of amino acid–formaldehyde by PMS. The rate law is (at constant pH)†

$$\frac{-d[PMS]}{dt} = k'_3K_1[AA][HCHO]_T[PMS] + k'_4\{[HCHO]_T - K_1[AA][HCHO]_T\}[PMS] \quad (15)$$

where $[HCHO]_T$ represents the total concentration of formaldehyde. If $K_1[AA] \ll 1$, then as an approximation $K_1[AA][HCHO]_T$ can be neglected in comparison to $[HCHO]_T$. Therefore

$$\frac{-d[PMS]}{dt} = k'_3K_1[AA][HCHO]_T[PMS] + k'_4[HCHO]_T[PMS] \quad (16)$$

† k'_i means k_i at constant pH.

Table 2. Kinetic parameters of formaldehyde catalysed oxidation of amino acids by PMS at 31° and $\mu = 0.25$

	Phenylalanine	Isoleucine	Threonine
k_b	2.60†	2.40	2.7†
($l^2\ mol^{-2}\ s^{-1}$)	2.30§	2.20§	2.7§
k_c¶ ($l\ mol^{-1}\ s^{-1}$)	0.06	0.06	0.07
$10^1 \times k_3K_1$ ($l^2\ mol^{-2}\ s^{-1}$)	9.4	8.3	7.7
$10^{-4} \times k_5K_1$ ($l^2\ mol^{-2}\ s^{-1}$)	9.0	97.8	123.8
$\Delta H^{\ddagger}$ (k cal mol^{-1})	14.4	14.4	14.6
$\Delta S^{\ddagger}$‖ (cal $deg^{-1}\ mol^{-1}$)	−27.4	−27.4	−26.5

† From amino acid variation, pH = 4.0.
§ From formaldehyde variation, pH = 4.0.
¶ k for the formaldehyde oxidation by PMS is 0.11 l mol^{-1} s^{-1} at pH = 4.0, $\mu = 0.25$ and t = 31°.
‖ pH = 4.0, $[HCHO] = 2.825 \times 10^{-3}$, [AA] = 0.03.

and

$$k_{obs} = k'_3K_1[AA][HCHO]_T + k'_4[HCHO]_T. \quad (17)$$

Comparison of Eq. (17) with Eq. (4) shows that k_b and k_c in Eq. (4) represent k'_3K_1 and k'_4 respectively. At constant $[HCHO]_T$, the plot of k_{obs} versus [amino acid] should give the value of $k'_4[HCHO]_T$ as intercept and $k'_3K_1[HCHO]_T$ as slope. From the intercept we can calculate the value of k'_4, the rate constant for the oxidation of formaldehyde. The values of k'_4, calculated from the three amino acid–HCHO systems are tabulated in Table 2 along with the value observed from the direct oxidation of formaldehyde by PMS.[9] Similarly from the slope of k_{obs} versus $[HCHO]_T$ plots, we can get the value of $k'_3K_1[AA] + k'_4$ and using the values of k'_4 obtained from the intercept in the plots of k_{obs} versus [AA], we can get k'_3K_1. The values of k'_3K_1 (k_b) are also tabulated in Table 2. Comparison of these values shows that the agreement is excellent for k'_3K_1 whereas the values of k'_4 (k_c) are less than the value obtained from the direct oxidation of formaldehyde. Similar observations were also noted in our earlier work.[2] This may be due to the fact that in our experiment we maintained only [AA] ≫ [PMS] and the concentration of HCHO is approximately equal to [PMS]. Strictly speaking the second term in Eq. (16) should not follow the pseudo-first-order kinetics and probably this may be the reason for the observed kinetics.

The effect of hydrogen ion is explained by considering the Eqs (9)–(12) and the rate law is

$$\frac{-d[PMS]}{dt} = (k_5K_1[AA] + k_6)[HCHO]_TK[HSO_5^-]\frac{1}{[H^+]} + (k_3K_1[AA] + k_4)[HCHO]_T[HSO_5^-] \quad (18)$$

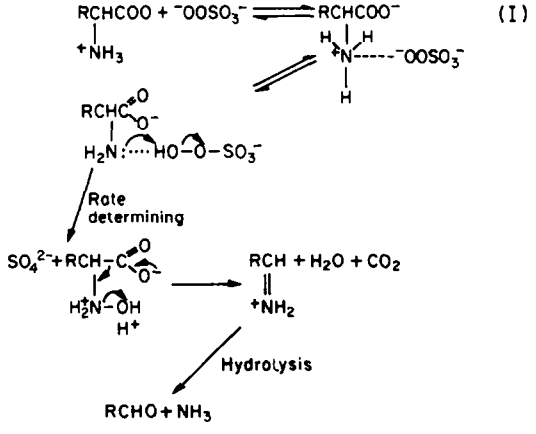
$$k_{obs} = (k_5K_1[AA] + k_6)[HCHO]_TK\frac{1}{[H^+]} + (k_3K_1[AA] + k_4)[HCHO]_T.$$

The plots of k_{obs} versus $1/[H^+]$ give $(k_5K_1[AA]+k_6)[HCHO]_TK$ as slope and $(k_3K_1[AA]+k_4)[HCHO]_T$ as intercept. The values of slope $(K[HCHO]_T)$ and intercept $([HCHO]_T)$ are, in this case, lower than the values of k_6 and k_4 from the oxidation of formaldehyde by PMS[9] (3.7×10^4 l mol^{-1} s^{-1} and 4.0×10^{-2} l mol^{-1} s^{-1}). This clearly proves that the rate constant for the oxidation of formaldehyde under this condition is always smaller than the rate constant obtained from the direct oxidation as discussed in the previous paragraph. As an approximation k_6 and k_4 can be neglected in Eq. (18) and the values k_5K_1 and k_3K_1 tabulated in Table 2 are only approximate. Here we have made an important assumption that the values of K_1 do not depend upon pH in the range we have studied (3.6–4.8). Fortuitous agreement of experimental observation with the rate equation justified this assumption.

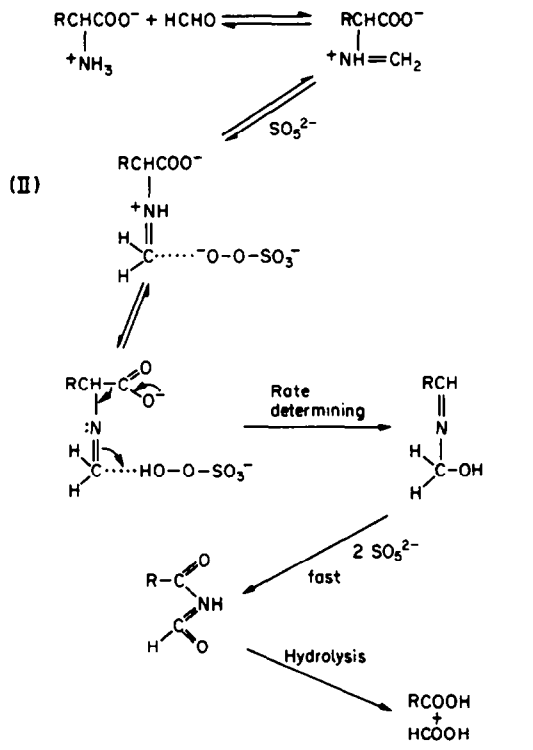
In all the cases the reactivity of SO_5^{2-} is several hundred times greater than that of HSO_5^- and this may be considered to be in favour of a nucleophilic attack by peroxide.[10] There is also abundant evidence that the peroxo anions are strongly nucleophilic as pointed out by earlier workers.[11,12] The foregoing kinetic results may suggest a nucleophilic substitution mechanism as shown below.

As pointed out earlier, aldehydes react with amino acids to give Schiff base compounds. The first step of the reaction of amino acid catalyzed by pyridoxal in both chemical and enzymic systems is the combination of the amino acid with the carboxyl group of the catalyst to form a Schiff base[5–7] which is represented as shown in Fig. A.

There is a larger positive charge on the Schiff base N atom in (2) than (1) and this provides an important additional driving force for electron withdrawal. The electron withdrawal towards the cationic N atom of the imine and into the electron sink of the pyridoxal ring from the α-C atom of the attached amino acid activates all three of the substituents on this C atom for reactions such as decarboxylation, transfer of amino group from an amino acid to an oxoacid etc., which require electron withdrawal from this atom. By comparison of the enzyme catalysed decarboxylation/deamination reactions of amino acids with our formaldehyde catalysed oxidation, we can assume that the oxidant reacts with the CH_2 group of $N{=}CH_2$ to give the activated complex II, which in the rate determining step



Scheme 1. Reaction scheme for the oxidation of amino acids by PMS.



Scheme 2. Reaction scheme for the oxidation of amino acids by PMS in presence of formaldehyde.

Fig. A.

rearranges to give the products. Equal values of $\Delta H^\ddagger$ and $\Delta S^\ddagger$ for all the amino acid–HCHO–PMS systems irrespective of the structure of the amino acids† supports our assumption that the reactions between PMS and amino acid–HCHO proceed through the same pathway for all the three systems. The production of aldehyde in any one of the intermediate steps can be eliminated as it would complicate the kinetics.

If the complex intermediate I is considered as the activated complex, we can explain the following observed facts in the oxidation of amino acids by PMS.

(i) Based on the inductive effect of the substituents we can expect that the order of reactivity should be as threonine > phenylalanine > isoleucine and $\Delta H^\ddagger$ values in the reverse order. Although the values of k_2 clearly bring out the relation, the observed values for $\Delta H^\ddagger$ are phenylalanine ≃ isoleucine > threonine. Probably this inductive effect may be responsible for the fact that in phenylalanine and isoleucine, SO_5^{2-}

† These $\Delta H^\ddagger$ and $\Delta S^\ddagger$ values are the same for the amino acid–HCHO–PMS systems reported in ref. 2. The amino acids include glycine, alanine, α-aminobutyric acid, valine, leucine and nor-leucine.

alone is reactive whereas in threonine both HSO_5^- and SO_5^{2-} are reactive.

(ii) If we consider the steric interaction in the formation of activated complex, the values of $\Delta S^\ddagger$ should be as isoleucine > threonine > phenylalanine. From our results we cannot compare the values of threonine with the remaining two amino acids since in threonine the oxidation by both HSO_5^- and SO_5^{2-} are taking place and the $\Delta S^\ddagger$ value is due to both reactions, whereas SO_5^{2-} alone interacts with phenylalanine and isoleucine. But, among phenylalanine and isoleucine the expectation is clearly brought out by the $\Delta S^\ddagger$ values.

(iii) One important observation not yet explained is the increase in k_{obs} after the conversion of a small amount of PMS in threonine alone. This can be very easily explained by the fact that the product aldehyde initially formed reacts with amino acid to give aldimine (Schiff base) which reacts more readily with PMS. In phenylalanine and isoleucine the product aldehyde probably cannot form the Schiff base. That is why these two amino acids show normal kinetics. Exceptional behaviour similar to isoleucine and phenylalanine was shown by valine in the oxidation of amino acids by PMS and this is attributed to the fact that the product aldehyde could not catalyse the reaction by the formation of Schiff base.[1] Probably this may be due to the steric factor, the resultant aldehyde from phenylalanine and isoleucine could not form Schiff base with the parent amino acid (phenylalanine and valine are found to form Schiff base with pyridoxal[13]). This fact is also confirmed by the observation that the product of oxidation of isoleucine and phenylalanine is aldehyde whereas threonine gives an acid.

(iv) Aminomalonate and α-methylaminomalonate undergo decarboxylation in the presence of pyridoxal derivatives at physiological temperatures.[14,15] This shows that the amino acids containing strongly electron withdrawing substituents catalyses the decarboxylation, probably due to an inductive effect. Based on this, the reactivity of SO_5^{2-} with the three amino acid–HCHO systems would be threonine > phenylalanine > isoleucine. Although the $\Delta H^\ddagger$ values for all the amino acid–HCHO systems are equal, the observed values for k_5K_1 are threonine > isoleucine > phenylalanine.

So far we have compared only β-phenylalanine, isoleucine and threonine. Let us compare these three amino acids with other amino acids reported in ref. 1. Suppose steric factors are alone responsible for the behaviour of valine as discussed earlier then isoleucine and threonine should behave similarly to valine in the oxidation by PMS since both amino acids have substituents other than hydrogen in the α-position to the $-NH_2$ group similar to valine. If the electronic effect (inductive effect) of the substituent is considered threonine should behave differently from valine and isoleucine since the former has an electron-withdrawing substituent. Comparison of the experimental results shows that threonine differs from valine and isoleucine and behaves like a normal amino acid, the product catalysing the reaction. Isoleucine and phenylalanine react with PMS similarly to valine. It is a well known fact that the increase in the electrophilicity of the carboxyl carbon increases the efficiency of Schiff base formation. The kinetic behaviour of threonine similar to other amino acids such as glycine, alanine etc. may be due to the inductive effect of the substituent (—OH group). Comparison of β-phenylalanine with other amino acids shows that β-phenylalanine should behave similar to alanine and glycine since the observed +I effect of the substituent is $CH_3 > C_6H_5CH_2$ as exemplified by the pK_a of acetic acid and phenylacetic acid. The observed kinetic behaviour of phenylalanine with PMS shows that phenylalanine behaves similarly to valine and this may be due to the fact that Schiff base could not be formed due to steric interaction as discussed earlier. Isoleucine behaves similarly to valine as expected because both are structurally similar.

The structural requirements for non-enzymic and coenzymic activity of vitamin B_6 (pyridoxal phosphate) analogues are the heterocyclic N for electron withdrawal, the 4'-formyl group and the phenolic OH group.[16] The outcome of this kinetic result is that in the non-enzymic model reactions, we can replace the heterocyclic nitrogen by an oxidant which will act as an electron withdrawer. This study also brings out the importance of Schiff base in the oxidative decarboxylation/deamination of amino acids in the presence of formaldehyde. Had it not been for the formation of Schiff base the observed rate constant should be of the order of oxidation of formaldehyde since the second order rate constant for formaldehyde oxidation is $\sim 10^3$ times greater than that of amino acids. However the observed rate at $\sim 10^5$ times greater than that of amino acids shows that probably, the rate constant for the oxidation of Schiff base is $\sim 10^2$ times greater than that of formaldehyde. Comparison of the values of k_b and k_c (Table 2) confirms this assumption.

EXPERIMENTAL

Potassium peroxomonosulphate was from the Du Pont Chemical Co., U.S.A. under the trade name "oxone". The purity of the triple salt $2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$ was found to be >95%. Peroxomonosulphate soln was prepared daily just before starting the experiments and the concentration was estimated by cerimetry using ferroin as indicator. Absence of free H_2O_2 was ensured by the test with permanganate. Amino acids were from the Loba-Chemie Indo Austranal Co. Other chemicals used were all of analytical grade.

Experiments were carried out in buffered media (AcOH—NaOAc) and a high concentration of the buffer (0.2 M) was maintained in the reaction mixture since the product HSO_4^- is a stronger acid than the oxidant HSO_5^-. No self decomposition of PMS was observed under our experimental conditions. Formaldehyde (S. Merck, India 30% soln) soln was prepared and estimated by the hypoiodite method.[17] Amino acid solns were prepared by gravimetry. The kinetics of the reaction were followed by iodometry at different time intervals.

The stoichiometry of the reactions was determined by taking a known excess concentration of PMS over amino acids and formaldehyde and allowing the reaction to completion at room temp. The concentration of formaldehyde was kept slightly greater than the amino acids throughout. Different ratios of amino acids, formaldehyde and PMS were taken and after the reaction was over, the remaining PMS was estimated by iodometry. Analysis of the results shows that the stoichiometry can be written as

$$\mathrm{R{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}{-}COOH + HCHO + 3\ PMS \rightarrow RCOOH + NH_3 + CO_2 + HCOOH}$$

$$\text{Threonine} + 2\ PMS \rightarrow RCOOH + CO_2 + NH_3$$

$$\left.\begin{matrix}\text{Phenylalanine}\\ \text{Isoleucine}\end{matrix}\right\} + PMS \rightarrow RCHO + CO_2 + NH_3.$$

Evolutions of CO_2 and NH_3 were detected by tests with lime water and Nessler's reagents respectively. In all the formaldehyde catalysed oxidations of amino acids, the formation of formic acid was detected by spot tests.[18] Phenylacetic acid, phenylacetaldehyde and threonic acid were confirmed by their physical properties and also comparison of the physical properties with authentic samples.

Acknowledgements—We wish to thank Prof. N. R. Subbaratnam for sparing the oxone sample and for his encouragement. We also express our gratitude to the referee for his critical comments and suggestions.

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